



NanoMagnetics Announces Doubling Of Nanoparticulate Storage Density

Thursday August 1, 2002

Company Press Release

SOURCE: NanoMagnetics Ltd.

NanoMagnetics announced today a new record areal density for nanoparticulate magnetic films. Many experts in magnetic recording believe that nanoparticulate films are the key to future areal density improvements, and will lead to disks that can store data at 100 times current levels. NanoMagnetics has made significant advances in its magnetic inks and film production techniques, leading to a raw areal recording density of just over 12 Gb/sq.in. This density was reached on commercial laptop-sized (65mm) glass substrates without the aid of an electronic channel or error correction, and would represent an even higher density within a disk drive. This achievement surpasses the previous record for nanoparticulate films of 6 Gb/sq.in. announced by NanoMagnetics in June of this year. In addition, NanoMagnetics has improved the uniformity of its film coatings and demonstrated high-density recording across more than 60% of a disk's surface.

This outstanding technical progress will be presented this month at The Magnetic Recording Conference (TMRC) in Santa Clara by Dr. Eric Mayes, the company's CTO and co-founder. In addition, Eric will be presenting at the storage industry tradeshow DISKCON in September. Eric said "A doubling in areal recording density in just over six weeks highlights the strength of this team and the promise of this technology. Also the support of Read-Rite and Seagate with their supply of recording heads is essential for our continued progress." CEO Brendan Hegarty said "Although aggressive, this rapid increase in our capabilities needs to continue in order to intersect, and then surpass the industry by the middle of 2003. We expect to have progressed to at least 25 Gb/sq.in. by year end, and we are currently in discussions with members of the industry as to possible partnerships."

NanoMagnetics is a private company focused on commercialising its rapidly expanding portfolio of nanoscaled materials, and in particular developing advanced magnetic materials for the information storage industry using its patented protein-based technology. These materials have the potential to replace the magnetic thin film technology used today by the hard disk industry, which is seeking to overcome the approaching physical "superparamagnetic" storage density limit. NanoMagnetics' technology offers a road map towards densities several orders of magnitude greater than are possible today, enabling terabyte drives for desktop PCs and handheld devices.

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PRINT READY

PFD

Nanometer-size structures fabricated by Bio-Nano-Process

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This is an abstract for a presentation given at the Ninth Foresight Conference on Molecular Nanotechnology. (Nov. 9-11, 2001 Westin, Santa Clara, CA)

Recently, nanometer-size structures have been attracting researchers' interests. A living thing, however, has already realized many kinds of nanometer-size functional structures. For example, the bacterial flagellar motor whose diameter is only 30 nm rotates at 10,000-100,000 rpm. Muscle is a linear motor and its 'engine' is around 20 nm. These nano-structures are made by self-assembly manner. This self-assembly is the key characteristic for building molecular machines and indispensable for handling nanometer-size molecules not only in the cell but also in the nanotechnology.

Some protein has the ability to accommodate inorganic materials, which called biominerization. Based on the protein's ability of self-assembly and biominearalization, we propose making inorganic nano-structures using protein supramolecules, which we name Bio-Nano-Process. As the first step, we fabricated two-dimensional array of quantum dots on a Si substrate using ferritin molecule.

Ferritin is a spherical protein supramolecule with a diameter of 12nm and composed of 24 hetero subunits. It has a 6 nm cavity which accommodates about 4,000 iron atoms *in vivo*. We employ a recombinant L-ferritin (1), composed of only L-type subunits from horse liver and its mutants. A two-dimensional crystal of iron-oxide loaded L-ferritin molecules which self-assembled at an air/water interface was transferred onto a hydrophobic Si surface (2). A well-ordered array of L-ferritin on the Si surface is observed by the high resolution scanning electron microscopy (SEM). L-ferritin shells were eliminated by heat-treatment under nitrogen or UV-ozone treatment, which left the array of nanometer size iron-oxide dots with little deformation. Fourier transform IR spectrophotometer (FTIR) analysis and XPS observation confirmed that the both treatments remove the protein shell completely. This well-ordered array of nanodot is suitable for quantum electronics key component.

We have also succeeded to load inorganic materials other than iron into ferritin cavity. For example Co, Ni, Mn, and CdS, which have different work functions. As the ferritin surface could be modified by short DNA sequence, some specific

combination of different kinds of ferritin with different material core would be realized. This gives a way to make groups of quantum wells with different energy levels and allows the design of more complex electronic circuits. The more precise and stronger interaction among ferritin molecules could be achieved modification of the ferritin surface by gene technology. With this method, we now try to make ferritin molecule dimmers and trimmers, each molecule of which has a different inorganic material core. This also leads to the complex nano-structures.

Our experimental results together with the idea of ferritin-ferritin interaction described above, it is expected that nanometer-size electronics circuits will be produced by this Bio-Nano-Process.

References

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Forbes

MAGAZINE

The Next Small Thing

Scientists are re-creating our world in the realm of the intensely tiny. The potential payoff: denser hard drives, smaller chips, better medicine.

Elizabeth Corcoran, *Forbes Magazine*
July 23, 2001

DEGREES OF SMALLNESS

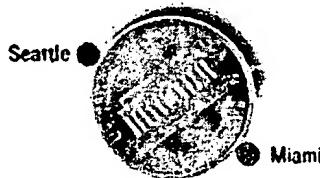
Science is venturing into a new borderland, one as mysterious as the deepest oceans and as foreign as intergalactic space. It is the realm of the tiny, where things are measured in an unimaginably small unit called a nanometer, a distance one-hundred-thousandth the width of human hair. In this place the distinctions we learned in high school between "squishy" biology and "hard" physics melt away. In nanotechnology everything is simply atoms. The scientist-explorers in this space are learning how to move those atoms and coax some to work with others, all in an attempt to change the very nature of matter itself.

Function follows form: Change the molecular structure of the materials used to make computer chips, for instance, and electronics could become as cheap and plentiful as bar codes on packaging. Lightweight vests enmeshed with sensors could measure a person's vital signs. Analysis of a patient's DNA could be done so quickly and precisely that designer drugs could be fabricated on the fly. A computer the size of your library card could store everything you ever saw or said.

If a nanometer were the size of a penny, a foot would stretch from Miami to Seattle.

In this fourth annual spotlight on innovators, *FORBES* has turned to the scientists laying the groundwork for this custom-made future. Nanotechnology is still in its early days. Our six pioneers are kindred spirits to those who produced crude solid-state electronics after the invention of the transistor or who roughed out the Internet in the early 1970s. "We're working out the rules of biology in a realm where nature hasn't had the opportunity to work," says Angela Belcher, a professor with the University of Texas.

Much of the work is aimed at building future electronic systems, whose manufacturing limits are becoming clear. Using established technology, such as lithography, the best that chip manufacturers can currently do is make circuit elements with dimensions measuring 130 nanometers across. The



prospect of building electronic systems that could be 1,000 times denser and significantly cheaper, bit for bit, is alluring. Redirecting chipmaking techniques for biology could dramatically improve how doctors diagnose and treat the ill.

None of this is easy, in no small part because scientists will be freely trespassing on one another's turf. To make such tiny electronic circuits, physicists are trying to mimic nature by finding ways to get inanimate materials to "self-assemble," in a fashion analogous to the way living things grow.

There are risks. Some nanotechnology tricks being investigated now will never be commercially feasible, and even the ones that will be could also attract the same sorts of hucksters that flocked to the Internet. Pundits even debate the possible unintended consequences of nanosystems, including whether nanomachines could spiral out of control.

The scientists profiled below hail from some of the world's top research organizations within both large companies and renowned universities. Their entrepreneurial spirit has pushed some to branch out to startups that aim to commercialize their work. The future will indeed be amazing. Watch these scientists make it so.

Forbes The Next Small Thing (continued)

Gerd Binnig, a fellow at IBM, remembers when other scientists would visit his laboratory in Zurich and angrily denounce him and colleague Heinrich Rohrer, as cheaters. "They just didn't believe us," he recalls. In 1981 Binnig and Rohrer built what they called a "scanning tunneling microscope." Painting snapshots of the surface of inorganic material, such as metal or semiconductors, the STM gave scientists their first visa to the nanoworld.

STICKY FACES

Random self-assembly



Programmed self-assembly



Like attracts like. Putting soldered dots on the faces of polymer blocks makes them "sticky," or naturally attracted to faces with matching patterns. Harvard University researchers "program" such blocks to self-assemble into specific shapes by designing those face patterns carefully. Adding transistors to the lattice results in a simple 3-D circuit.

By bringing a metal probe within two atoms of a sample material, Binnig and Rohrer showed that the material's surface was as bumpy as the Rocky Mountains instead of smooth and uniform, as most previous calculations had assumed. Even Binnig could hardly believe their results. I thought matter was simpler. I was impressed by its beauty.

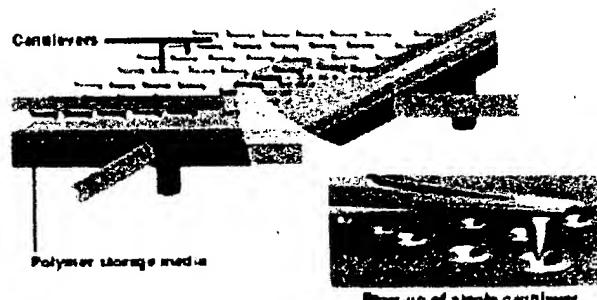
As soon as scientists could see individual atoms, they wanted to start playing with them. (The work also won Binnig and Rohrer a share of a Nobel Prize in 1986.) Fast-forward to the present: Rohrer, 68, is retired, but Binnig, 54, is breaking new nano-ground — literally — with his millipede storage system that can record more than 400 gigabits of data over a square inch. That's a 50-fold step up from IBM's densest commercial drive, a 70-millimeter platter that packs 26 gigabits.

Along with a team of IBM colleagues in Zurich, Binnig created a nanoscopic brush with 1,024 tiny tips, each attached to its own cantilever. As the cantilevers move up and down, they make dents in a plexiglass-like polymer; picture an inverted version of the bumps on a roll of music for a player piano designed to charm an amoeba. The tips can also read the dents back, at rates of up to 100 megabits per second, by brushing back over them.

To start over again, heat the polymer and the dents disappear as the molecules realign themselves. Our intention was to demonstrate that nanotechnology isn't that far out, declares Binnig.

It will take IBM two to three years to commercialize the millipede storage system, Binnig expects. That the millipede works at all surprised even Binnig, who says he procrastinated building the device because he thought it wouldn't work. In the nanorealm your intuition fools you, Binnig says. You might think it would not work but it does.

BUMPY STORAGE



IBM's "millipede" stores bits by making nanoscale indentations in a polymer using 1,024 tiny tips, attached to cantilevers. Heating the polymer "erases" the data by rearranging the molecules in the polymer so that the material is smooth again.

At the heart of Binnig's work are mechanical principles. Others are turning toward nature for insights into how to manipulate matter. Nature builds

nanostructures all the time. But rather than painstakingly trying to assemble every feature, biological systems essentially are programmed with rules that guide how materials should build themselves.

Chemists work much the same way, says George M. Whitesides, 62, who joined Harvard University as a tenured chemist in 1982. "Chemists have always been in the business of taking atoms and putting them together with other atoms with precisely defined connections," he says.

That kind of work used to be aimed at concocting new drugs. Now it's aimed at the microchip business. By the 1980s microelectronics engineers were beginning to worry that packing ever more transistors onto computer chips was going to get impossibly expensive. Whitesides, who had long served as a consultant to the Department of Defense, had a reputation for doing unconventional chemistry by exploring interactions between unlikely pairings of materials. Why not, he asked, put chemistry to work to make novel electronic circuits?

That question has led Whitesides to the forefront of one of the most intriguing nano-explorations: figuring out how to coax materials to "self-assemble," or put themselves together in precise ways.

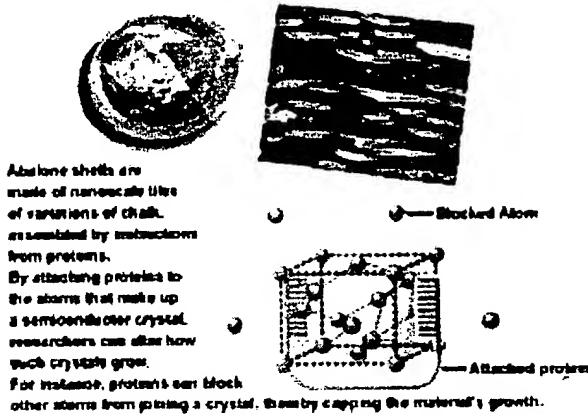
Trying to build nano-scale electronics with even the most sophisticated tools from the chip business is exquisitely hard-like doing a tiny line drawing with an infant's extra-thick

crayons. By contrast, living cells are full of machinery that assemble nanoscale structures all the time.

Forbes The Next Small Thing (continued)

Inspired by the complex structure of proteins, Whitesides thinks he can build a three-dimensional circuit that assembles itself. He takes tiny polymer blocks and affixes electronic devices like transistors on a number of faces. His team adds solder dots on other faces, then strings the blocks together like beads on a fine wire.

TILE BY TILE



The "magic" is in the solder dots, which are attracted to one another, much the way two drops of water on a windshield merge into one. The die sides with, say, two solder dots are attracted to others with two solder dots; those with three are attracted to other groups of three.

Suspend the necklaces of blocks in water and the solder dots come together, forming connections and, ultimately, a three-dimensional circuit. "If we do the design right, this thing should fold together just one way," Whitesides says.

Making, say, 500 components come together is daunting, he adds. "But we're not steering blind. Nature does it. We know that 500 amino acids can fold to make just one protein."

Angela Belcher, 34, of the University of Texas, also believes that proteins are the keys to unlocking new semiconductor materials.

Abalone, she says, make their shells by creating a fine brick-like wall of

nanosize tiles of two types of chalk, something that makes them 3,000 times tougher than the chalk found in rock. "They're perfect nanostructures, and that's related to why they're so tough," she says. Proteins signal when the abalone should grow a tile. As Belcher was finishing her graduate degree, she made a conceptual leap:

Why not use proteins to direct the nanostructure of semiconductors? "People didn't think about putting these things together," Belcher says. "Most just thought, 'This will never work.'"

Structure matters. For decades, silicon engineers have worked hard to make silicon crystals grow as perfectly as possible so that elec-

trons will whiz through, producing a faster chip. Belcher wants to control far more aspects of crystal growth. She did not know which proteins might trigger semiconductor growth. So she turned to a time-tested approach from the pharmaceutical industry: Throw the kitchen sink at 'em.

She started with a billion viruses, each carrying a different genetically modified protein. She mixed them in solution with a semiconductor, then checked to see whether some proteins would stick to the semiconductor. The technique worked. She washed away

the irrelevant ones, then repeated the process. By running through the routine a half dozen times, Belcher began to believe that she could control how semiconductor crystals grow.

Now, two years later, Belcher and her team are collecting a toolbox of proteins that alter crystal growth in various ways. "It took the abalone millions of years to evolve," Belcher says, to get the right proteins. "It takes about three weeks on the bench top."

Working through the periodic table, Belcher's university team has tested proteins that stick to about 20 different inorganic materials. This spring Belcher and Evelyn Hu of the University of California, Santa Barbara, cofounded a company, Semzyme, to systematically develop a protein toolkit. "Right now, we're trying to learn the rules," she says.

Harold Craighead, 48, recently appointed dean of engineering at Cornell University, is trying hard to break the rules—at least, the traditional rules that separate fields like physics and biology. At Cornell's Nanobiotechnology Center physicists, engineers, biologists and chemists collaborate, crushing the time-honored academic distinctions separating them. He tries to do the same in his own work as a scientist, using a physicist's collection of tricks to understand and work with biological molecules. He pokes, prods and stretches DNA molecules to understand the physics that govern them.

CONTRARY THINKING

Cross section of channel

Direction of DNA motion →

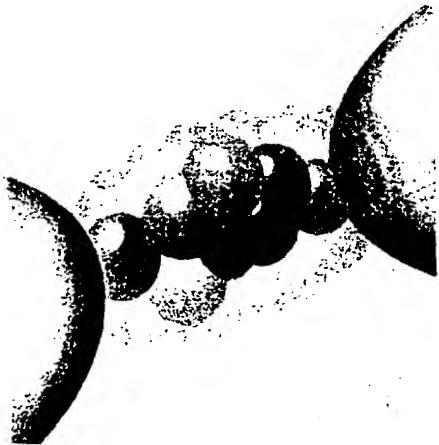


Separating large fragments of DNA from a solution takes time. At Cornell University scientists design a network of tiny channels laced with nanometer-size obstacles. When they apply an electric field, forces pull bigger DNA molecules along faster than small ones.

Forbes The Next Small Thing (continued)

One result: a technique for dramatically speeding up the job of separating large fragments of DNA, a likely boon for diagnosing diseases.

MOLECULAR CONNECTION



Gold dots are the electrodes at both ends of a molecular switch designed at Yale University. Applying a voltage makes the molecular conduct electricity, turning it "on".

To study the properties of DNA molecules, Craighead and his associates employ lithographic equipment like that used by the chip industry to make tiny obstacle courses for molecules. On a piece of silicon, they etch tiny channels with bumps 35 to 90 nanometers high. They then put a drop of solution containing DNA fragments at one end of the channel and turn on an electric field: weirdly enough, the bigger the fragments, the faster they move over the bumps. It takes only a few minutes for Craighead's group to separate large DNA fragments from a sample. Traditional means could take many hours for the same material.

Marrying molecular biology and lithography is already producing a wealth of other novel diagnostic techniques. One of Craighead's favorites: a tool that could detect whether food is spoiled. It's the micron equivalent of a dipstick, a tiny cantilever with antibodies on the tip. The antibodies attach to certain bacteria, causing the cantilevers to vibrate. Electronic systems sense the vibrations and set off an alert. With such nanoscopic feelers, they can detect even a molecule of gas.

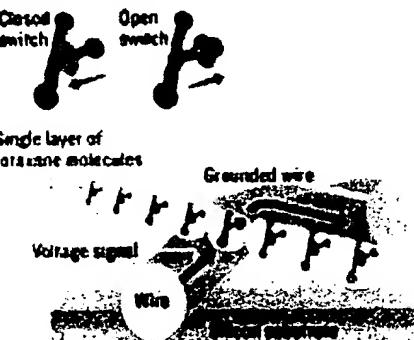
"This is no more sophisticated than the electronics in a singing birthday card," Craighead says. Such systems could easily be built into every refrigerator.

R. Stanley Williams, 49, and a fellow at Hewlett-Packard, just wants to build a better computer. "A computer still isn't smart and isn't easy to use," Williams says. "We want to make vastly more capable computers that are cheaper to build and that run on less power."

The model Williams and his colleagues have in mind is, of course, the human brain, an instrument with trillions of neurons. Any one neuron is slower to act than a transistor on a Pentium chip, but collectively, the brain's neurons process far more information than the biggest array of Pentiums.

To mimic the brain Williams needs switches and wires thousands of times smaller than those in the best silicon devices and a radically different design for the overall computer. For switches Williams and his HP colleagues, along with James Heath at the University of California, Los Angeles, are turning to molecules. Carbon nanotubes are promising candidates for wires. They are atomically perfect structures, 1 to 2 nanometers wide, and as much as a million times as long. Some are metallic and have proven to be the best conductors yet discovered, others are semiconductors.

SWITCHING PLACES



A molecular switch designed by researchers at UCLA and Hewlett-Packard sandwiches a single layer of switch molecules between layers of nanoscale wires. Applying different voltages turns the switches either on or off.

For their system Williams and his colleagues construct an electronic sandwich: a layer of wires, then a layer of rotaxane molecules that act like switches, topped with another layer of wires that run perpendicular to the first layer. The molecules become devices at the points where the wires cross.

Some of the molecular switches may not work--something that would be a devastating problem for conventional electronic circuits. But the scientists believe they can live with defective components by programming their way around them, a trick that has been used in some kinds of silicon chips and in experimental computers.

That's the future. For now Williams and his team are tackling such fundamental challenges as showing that their switches work and figuring out how to get data in and out of tiny molecular devices.

At UCLA Heath is nearing the end of a two-year effort to build a molecular 16-bit memory unit. "By the end of summer we'd like to demonstrate, on a nanometer scale, logic devices that are like the chips you could buy in 1970," Williams says. "Then we'd like to boost performance eight times every 18 months," he adds, in order to achieve a 16-kilobit memory by 2005. If he is successful, he reckons that in ten years his devices will rival the power of the best available conventional silicon chips.

Yet because his chips will be built from organic molecules, manufacturing them might be more like rolling out a sheet of photographic film than etching squares of silicon, Williams says.

Mark Reed, 46, now chairman of Yale University's electrical

Forbes *The Next Small Thing (continued)*

engineering department, is convinced that some form of electronics will ultimately be built from self-assembling components. In the beakers in his laboratory he is exploring just what types of molecules can be coaxed to work together to conduct current.

In June Reed published a paper describing how he made memory devices by sandwiching carbon-based molecules between two dots of gold measuring less than 50 nanometers across. Apply a voltage to the device and the molecules rearrange themselves to conduct, essentially turning on. Other pulses can nudge the molecules to rearrange themselves into a nonconducting state, turning off much as a random access memory chip does. Still other voltage pulses can read whether the molecules are off or on.

"Our next step is to get our hands on the knobs of the controls," says Reed, by understanding the physics behind how electrons move through such materials. "Molecular devices could be used to replace memory. Will they be as good as silicon? Probably not. Will they be cheaper? Yeah."

Imagine, he says, memory so cheap that people throw away electronics the way they do packaging. When electronics are that cheap, the way we use them changes fundamentally. Electronics, for instance, could replace bar codes. Shoppers would still pick groceries off the shelf, but rather than waiting in a checkout line, they would wheel their loaded carts through a portal like a metal detector. The electronics would signal how much should be deducted from the shopper's bank account.

Nice — yet Reed contends that the most intriguing applications have yet to be invented. "If you had extrapolated from 1950s technology, we'd all have nuclear-powered toasters instead of PCs on our desks."



In an experiment done in George Whitesides's lab, 20-micrometer-wide gold plates stack themselves in arrays of columns.

SELF-ASSEMBLY

Devices that build themselves are key to nanotech

As researchers begin trying to build devices and novel materials at the nanoscale (a nanometer is a billionth of a meter, the size of a few atoms), they're facing a massive challenge. While it's proving possible, in many cases, to push molecules around to form tiny structures and even functioning devices, efficiently mass-producing anything with nanoscale features is another matter altogether. But what if millions of these nano building blocks did the heavy lifting and assembled themselves into the desired structures—avoiding the use of expensive and elaborate manufacturing instruments?

Self-assembly has become one of the holy grails of nanotechnology, and scientists in numerous labs are working to transform it into an effective nano engineering tool. In some sense self-assembly is nothing new: biology does it all the time. And for decades, scientists have studied "supramolecular" chemistry, learning not only how molecules bind to one another but how large numbers of molecules can team up to form structures; in fact, the concept of self-assembly largely grew out of chemists' attempts to make molecules that aggregated sponta-

neously into specific configurations, in the same way biological molecules form complex cell membranes.

But now, with an expanding understanding of how molecules and small particles interact with one another, researchers can begin to predict how such elements might self-assemble into larger, useful structures like the transistors on a semiconductor chip. "Self-assembly provides a very general route to fabricating structures from components too small or too numerous to be handled robotically," says George Whitesides, a chemist at Harvard University and pioneer in the field.

To better understand how self-assembly works, Whitesides and his coworkers have recently shown that selectively coating the surfaces of microscopic gold plates with a sticky organic film can, under the proper conditions, trigger thousands of such plates to self-assemble into three-dimensional structures. So far, Whitesides's team has created a relatively large functional electronic circuit using a similar technique. The next step will involve shrinking the circuit to the micrometer scale, creating more complex three-dimensional structures out of silicon. While micrometer-sized electronic components are nothing new—Intel makes

them all the time—Whitesides's experiments could provide valuable clues as to how to better manipulate self-assembly.

Nature itself is also providing scientists with a model of how to create self-assembling electronic devices. Materials scientist Angela Belcher at the University of Texas at Austin sorted through billions of different proteins to find ones that recognize and bind to different types of inorganic materials. For instance, one end of the protein might bind to a specific metal particle and the other end might stick to the surface of a semiconductor such as gallium arsenide. Given the right prompts, the proteins could direct nano-sized particles of inorganic materials to form various structures.

This past spring, Belcher cofounded a company called Semzyme that plans to create a library of these protein-mediated building blocks. They could have any number of technological applications, in making such things as biomedical sensors, high-density magnetic storage disks or microprocessors.

Chemists at labs such as those of Hewlett-Packard, the University of California, Los Angeles, Yale University and Rice University are also attempting to develop self-assembled molecular computers. If they succeed, however, it will take years.

Meanwhile, less ambitiously, other researchers are making rapid strides in using self-assembly to build increasingly complex—and increasingly small—three-dimensional structures that could be compatible with existing devices. For instance, certain features of a disk drive, like the storage medium, could be created using self-assembly, while larger components needed to connect the device to the outside world would be made using conventional techniques. "We hope that self-assembly will be able to inexpensively replace certain stages in the production of materials and devices, where control is needed at the molecular level," says engineer Christopher Murray of the nanoscale-science division of IBM Research in Yorktown Heights, NY.

If he's right, nano engineering will get a whole lot easier. —Philip Ball

PDF

Nanometer-size functional structures made by self-assembly of protein molecules

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This is an abstract for a presentation given at the
Seventh Foresight Conference on Molecular Nanotechnology (October 15
-17, 1999 at the Westin – Santa Clara, CA)

Life is supported by highly sophisticated organisms composed of assemblies of proteins and nucleic acids, which work as molecular machines. Molecular machines show many interesting characteristics, among which are self-assembly and highly efficient energy transduction. Self-assembly would also be essential in engineering application, when one would try to handle molecules for nanometer-scale fabrication. Self-assembling proteins provide a way to realizing important break through in making nanometer-size devices or functional structures based on principles completely different from what the present technologies are base on.

As a trial of fabricating nanometer-size functional structures, we employed ferritin particle assembly to make nano-dots for quantum electronic devices. Ferritin consists of a protein shell with a diameter of 12 nm, which surrounds the core of Fe_2O_3 with a diameter of 6 nm. It is possible to make two dimensional crystals of protein molecule on a Si wafer using the method by Furuno *et al.* (Thin Solid Films 180, 23-30, 1989). We treated ferritin crystals on Si wafer at around 400 C in a nitrogen atmosphere. This treatment burned out the protein shell and yielded two dimensional arrays of inorganic iron oxide dots on the Si wafer. The size and repeat distance of the dots were 6 and 12 nm, respectively, as measure by FE-SEM and AFM. As the diameter of the iron oxide dots is only 6 nm, this two dimensional array of inorganic iron oxide dots has a potential to be used as quantum dots. Feasibility study of the application of this dot array to the structure of semiconductor memory is now in progress.

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P.D.

Icosahedral Virus Particles as Addressable Nanoscale Building Blocks

Qian Wang, Tianwei Lin, Liang Tang, John E. Johnson*, and M.G. Finn*

Dedicated to Professor K. Barry Sharpless

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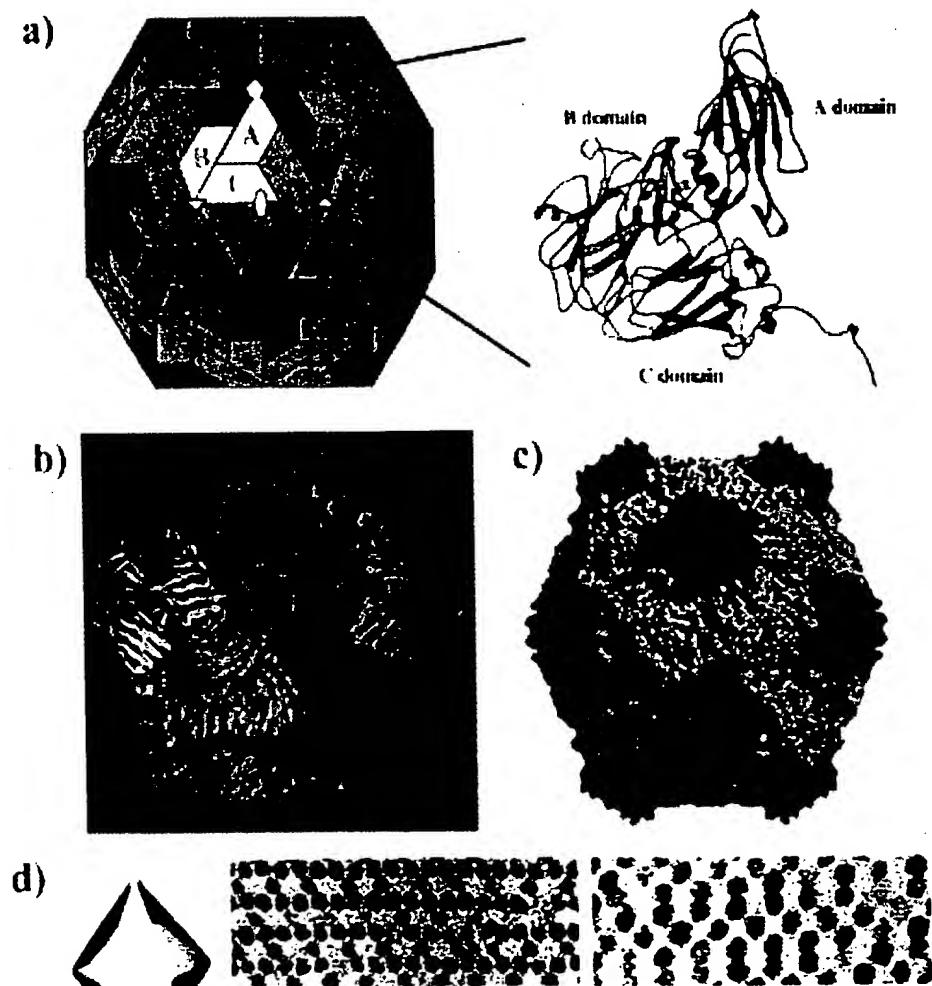
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Nanochemistry is the synthesis and study of well-defined structures having dimensions of 1-100 nanometers (nm), spanning the size range between molecules and materials.^[1] While supramolecular chemistry (making small molecules bigger) and microfabrication techniques (making big structures smaller) attack from the flanks, biology employs many constructs of this size. Examples include the photosynthetic reaction center, the ribosome, and membrane-bound receptor-signaling complexes, all notable because of their sophisticated yet modular function. The burgeoning field of nanotechnology^[2] seeks to mimic the information-handling, materials-building, and responsive sensing capabilities of biological systems at the nanometer scale. The special requirements of this enterprise would be well served by building blocks of the proper size with predictable and programmable chemistry.

Cowpea mosaic virus (CPMV) particles are 30 nm-diameter icosahedra, formed by 60 copies of two different types of protein subunits (Figure 1A).^[3] The physical, biological, and genetic properties of CPMV have been well characterized.^[4] Approximately one gram of virus is easily and routinely obtained from a kilogram of infected leaves of the black eye pea plant. The structure of CPMV has been characterized at 2.8Å resolution by X-ray crystallography and an atomic model of the particle has been constructed.^[5] The virion displays icosahedral symmetry to the resolution of the crystal structure and an infectious clone of the virus allows site-directed and insertional mutagenesis to be performed in a straightforward and rapid manner.^[6] The particles are remarkably stable, maintaining their integrity at 60 °C (pH 7) for at least one hour and at pH values from 3.5 to 9 indefinitely at room temperature. Different crystal forms of the virus can be readily produced under well-defined conditions (Figure 1B).^[7, 8] In this report we describe the selective chemical derivatization of the native virus and the crystal structure of a derivatized particle as well as the preparation of site specific mutations that allow the attachment of fluorescent dyes and gold clusters through maleimide linkers. The results of these studies demonstrate that the virus particles can be exploited as addressable nanoblocks imbued with a variety of chemical and physical properties.^[9]

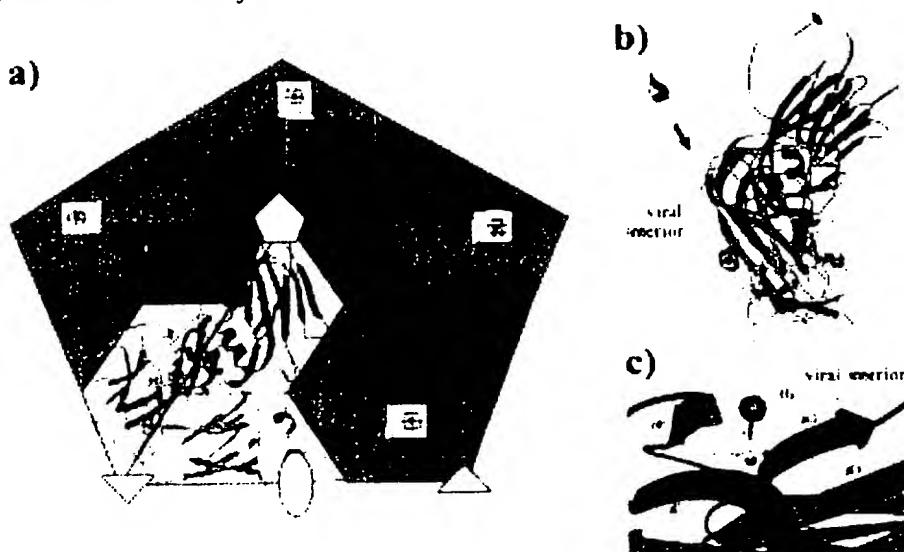
Figure 1. Structure of cowpea mosaic virus and its crystals. (A) (left) A diagrammatic representation of CPMV showing the distribution of the two subunits that comprise the "asymmetric unit", 60 copies of which form the icosahedral particle. The trapezoids in red and green represent the two domains of the large subunit clustered around the 3-fold symmetry axes and the blue trapezoid represents the small subunit clustered about the 5-fold symmetry axes. (right) The folds of the two subunits. (B) Organization of five asymmetric units into the "pentamer" centered around a small hole at each 5-fold axis. (C) Representation of the X-ray crystal structure of CPMV, highlighting the EF-loop (in red) in the large subunit in which the cysteine-containing insert is made. (D) A hexagonal crystal of CPMV (left). Electron micrographs of crystals thin sectioned perpendicular to the c axis (middle) and the a axis (right) showing the remarkably open lattice. Previous studies have shown that proteins with dimensions in excess of 50 Å can be reversibly soaked into the crystals. A typical crystal contains 10^{13} particles.



Thiol-selective chemical reagents were used to probe the reactivity of non disulfide-linked cysteine residues in the native virus particle. The crystal structure shows that CPMV has no free sulfhydryls on the outer surface^[5] and there was no evidence of reaction with the commercially available monomaleimido-Nanogold® reagent (Nanoprobes, Inc., Yaphank, NY) having a molecular diameter of 1.4 nm. However, adducts of the native virus with ethyl mercury

phosphate (EMP), an agent with a strong affinity for free sulphydryls but with a dimension of only a fraction of a nanometer, were readily formed. The resulting labeled virus was crystallized under conditions previously employed.^[7] Crystals with rhombic dodecahedral morphology, identical to those obtained with native virus, were obtained in 4 days and X-ray diffraction data to 6 Å resolution were collected on beam line 11-1 at the Stanford Synchrotron Radiation Laboratory. Figure 2A shows a pentamer of the icosahedral constellation of EMP molecules visualized in a difference electron density map. Figures 2B and 2C show that the EMP reacted with a single CYS residue at position 295, on the *interior* surface of the large subunit. This residue contains the sulphydryl group that appears in the X-ray crystal structure of CPMV to be the most exposed to solvent.^[10]

Figure 2. Crystallographic analysis of CPMV particles derivatized with ethyl mercury phosphate. Amplitudes in the Fourier series calculation were obtained by subtracting structure amplitudes computed from the atomic model of native CPMV from the measured structure amplitudes of the EMP derivative. The difference amplitudes and native phases were used to compute electron density. (A) The pentameric assembly of CPMV protein about the five-fold symmetry axis. The difference electron density map reveals bound EMP molecules to be located solely at a single position below the outer capsid surface corresponding to CYS295; five such sites are shown here. (B) A view showing the fold of the CPMV asymmetric unit with EMP difference density. (C) A close-up view showing the position of the EMP difference density.



The reactivity of native CPMV toward an organic thiol-selective reagent was found to be different than toward mercuric ion. Thus, wild-type CPMV was condensed with 5-maleimidofluorescein (**1**); Figure 3 shows a plot of the number of molecules of dye attached to

native CPMV as a function of increasing concentration of the dye reagent.^[11] The curve plateaus at 60 attached dye molecules per particle, suggesting that a single cysteine residue per icosahedral asymmetric unit is most reactive. Denaturing gel electrophoresis analysis of the derivatized protein (Figure 3) showed that the dye is attached exclusively to the *small* subunit, not to the large subunit as was the case for reaction of EMP at CYS295. This alkylation reaction must also occur on the interior capsid surface (see below), but its exact position has not yet been established. Multiple interior cysteine sites, on both small and large subunits, can be addressed with **1** at higher dye to subunit ratios without damaging the structural integrity of the particle (data not shown). Small molecules such as **1** are thus apparently able to diffuse through the capsid, perhaps through the small hole that appears in the crystal structure at each 5-fold symmetry axis (Figure 1B).

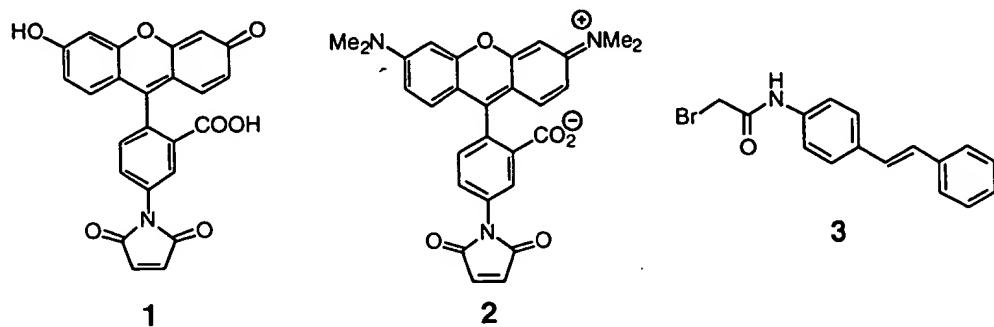
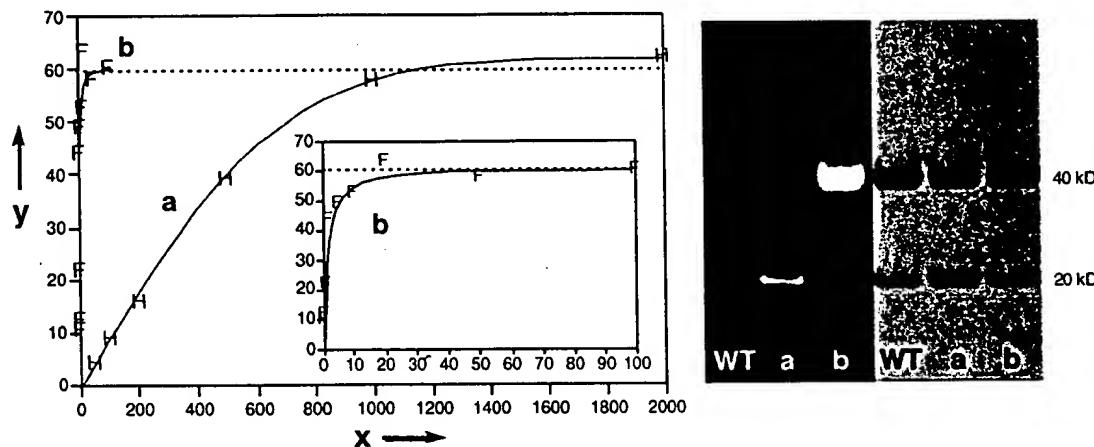


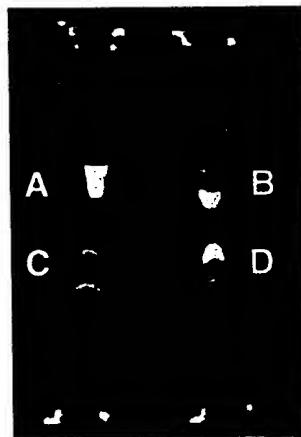
Figure 3. (left) Plots showing the number of the covalently attached fluorescein molecules to native CPMV (a) and the CYS insertional mutant virus (b) as a function of increasing ratio of reagent 1 to virus (x = equivalents dye-maleimide used per asymmetric unit; y = dyes attached per particle). Stoichiometries were determined by absorption measurements on solutions of labeled particles by comparison of the intensities of the dye (494 nm) to protein (260 nm), each having non-overlapping bands with well-established molar absorptivities. (right) SDS-PAGE analysis: (WT) wild-type CPMV; (a) wild-type CPMV-1 conjugate having approximately 17 dye molecules attached per capsid; (b) mutant CPMV-1 conjugate containing 60 dyes per capsid. On the left (black background) is the gel visualized directly under ultraviolet light, showing the fluorescein emission localized in the small protein subunit of the wild-type virus and the large subunit of the mutant. On the right (light background) is the gel visualized after Coumassie blue staining, showing both small and large subunits.



Mutant CPMV particles were prepared in order to display sulphhydryl groups on the exterior surface of the structure. Thus, a five-residue insertion containing cysteine (GGCGG) was placed between positions 98 and 99 in the large subunit (Figure 1C). Yields comparable to wild type were obtained with this virus, but the presence of β -mercaptoethanol or tris(2-carboxyethyl)phosphine was required throughout the isolation procedure to avoid crosslinking of particles when they were pelleted by ultracentrifugation or stored after purification. These particles were reacted with increasing concentrations of fluorescent dye, with the results shown in Figure 3. In the presence of smaller quantities of the dye-maleimide reagent, up to 60 dye molecules were attached per CPMV virion at significantly faster rates than were observed with the native particle. The reactive interior CYS residue remains active in the mutant particle, such that the two positions can be addressed sequentially under controlled conditions. Thus, reaction with 50 equivalents of 1, followed by purification and reaction with 1000 equivalents of 5-maleimide tetramethylrhodamine (2), gave CPMV decorated with an average of 55 fluoresceins and 49 rhodamines per particle, as determined by UV-vis absorbance spectroscopy.^[11]

The differential properties of the reactive cysteine residues of wild-type vs. the mutant virus were probed using the stilbene derivative **3** and antibodies to the stilbene moiety recently developed (Figure 4).^[12] Since the bromoacetamide group of **3** is selectively reactive with cysteine sulphydryl groups under conditions similar to those used for maleimides, CPMV-stilbene conjugates were readily prepared and purified for native and mutant virus particles. When treated with antibody 19G2, the presence of an antibody-stilbene complex is revealed by blue fluorescence upon excitation with a hand-held ultraviolet lamp; no fluorescence is observed in the absence of either stilbene or antibody. Figure 4 shows the results for intact and denatured CPMV conjugates of **3**. The successful attachment of the stilbene group to both wild-type and mutant CPMV was shown by the appearance of strong fluorescence when the denatured samples were treated with the antibody. The mutant CPMV-**3** conjugate similarly showed antibody binding to stilbene on the intact particle, but the wild-type CPMV-**3** conjugate did not. This is consistent with the attachment of **3** to the interior capsid surface of the wild-type virus, where it is inaccessible to the indicating antibody. In contrast, the mutant virus displays its stilbene-decorated cysteine residues to solvent on the exterior surface.

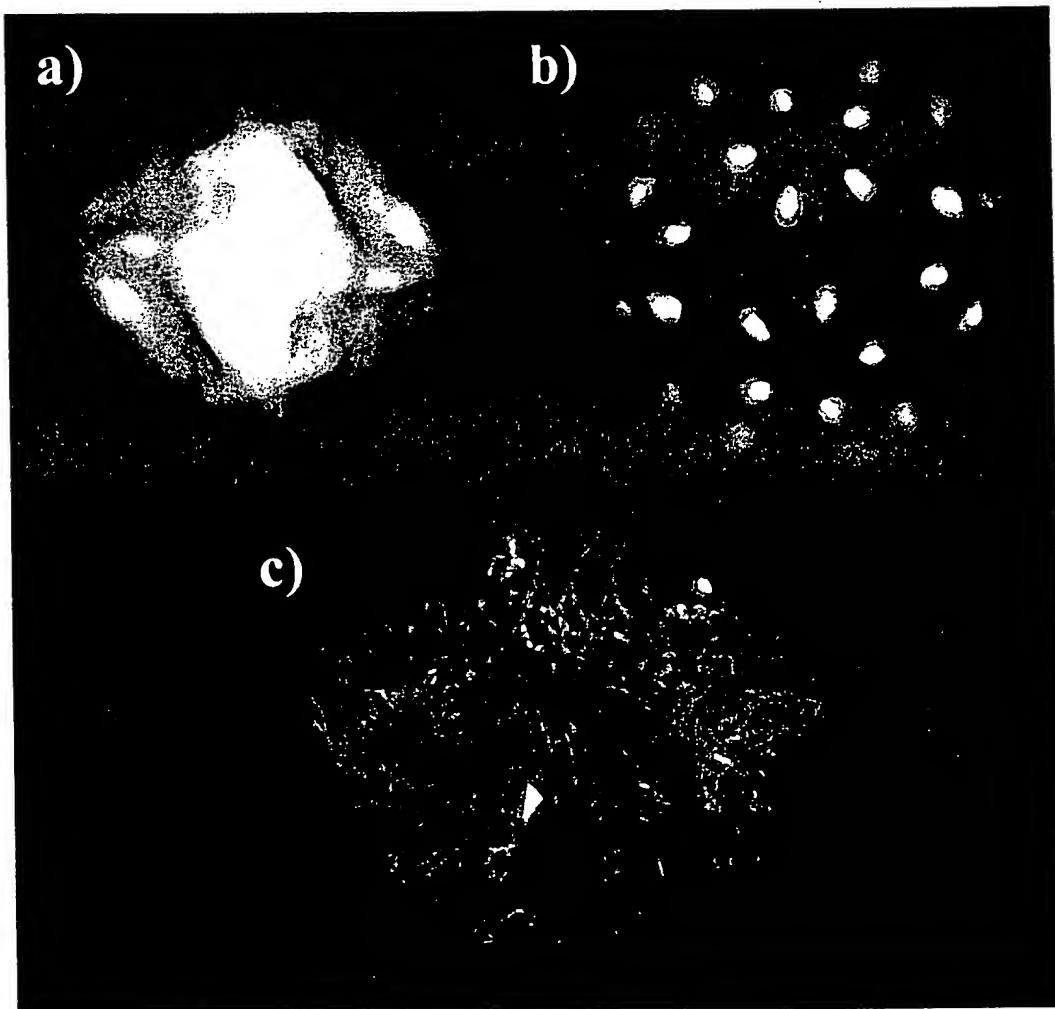
Figure 4. Black-and-white photograph of samples under ultraviolet irradiation: (A) mutant CPMV-**3** conjugate + stilbene-binding antibody 19G2; (B) denatured mutant CPMV-**3** conjugate + 19G2; (C) wild-type CPMV-**3** conjugate + 19G2; (D) denatured wild-type CPMV-**3** conjugate + 19G2. Note that denaturation causes precipitation of the viral protein, but exposed stilbenes are still recognized by the antibody.



The mutant virus was also reacted with monomaleimido-Nanogold[®], providing virions displaying absorbance at 420 nm, indicating attachment of the gold cluster. Derivatized particles

were flash-frozen and examined by electron microscopy and a three-dimensional image reconstruction was computed (Figure 5A).^[13] Figure 5B shows a difference map in which density for the model CPMV structure was subtracted from the density computed in the image reconstruction. The gold particles are clearly visible at the positions of the inserted cysteine residues (Figure 5C), providing an example of the installation of chemical structures at designated positions on the icosahedral protein template.

Figure 5. Electron cryo microscopy analysis of derivatized CPMV CYS mutant. (A) Three-dimensional reconstruction of CPMV particles at 29 Å resolution labeled with 1.4 nm gold particles. (B) Difference electron density map generated by subtracting density computed with the native CPMV X-ray structure from the density shown in Figure 5A. Since the computed native CPMV density was made from only protein, the nucleic acid (shown in green) is visible in the difference map as well as the gold particles. (C) A pentameric section of the difference electron density map around the five-fold symmetry axis superimposed on the atomic model of CPMV showing that the gold is attached at the site of the CYS mutation.



We have shown here for the first time that a virus can function as a convenient and programmable platform for organic chemical reactions. Derivatized CPMV particles will generally display 60 copies of the attached molecule, making these systems analogous to very large dendrimers.^[14] High local concentrations of the attached chemical agent are thereby engineered in the vicinity of the particle, which may result in novel chemical and/or biological properties. Appropriate choices of derivatizing agents can selectively target residues on the inner or outer surface of the particle, allowing double labeling of the particles with different molecules. We are continuing to expand the range of reactivity of CPMV with different mutational insertions and chemical derivatizing agents, with the goal of engineering novel function within a single particle and for aggregates of particles. For example, the virus surface can be patterned with metal nanoparticles if multiple cysteine residues are placed at accessible positions. Such a pattern could potentially be used to form a conducting "wire" at the nanometer scale. Furthermore, CPMV icosahedra show a propensity for self-organization. Straightforward crystallization procedures lead to well-ordered arrays of 10^{13} particles in a typical 1mm^3 crystal, which may be regarded as a meso scale self-organization of nanoblock components.^[15] Thus, we believe that CPMV and other viruses have a rich future in applications spanning the worlds of molecular, biological, and materials science.

Experimental Section

Typical procedure for chemical derivatization of CPMV. Virus (1 mg/mL in buffer) and maleimide reagent at the indicated concentration were incubated in an 80:20 buffer:DMSO mixture at 4 °C for 6 to 48 h; all reactions contributing to Figure 3 were run for a constant time of 24 h. (Unless otherwise noted, "buffer" refers to 0.1M potassium phosphate, pH 7.0.) Reaction mixtures were purified on small scale (80 μL) by passage through short size exclusion columns prepared with Bio-Gel® P-100 Gel (Bio-Rad) in buffer, packed in Bio-Spin® disposable chromatography columns, and eluted at 1000 gravities for 3 minutes. Multiple passages through freshly-packed columns were performed until dye was undetectable in the wash solution and the relevant absorbance ratios for the virus samples were constant. Purification of larger quantities of

derivatized virus was performed by repeated ultracentrifugation pelleting and resuspension in buffer. Yields of labeled virus particles after purification were 70-80%. For quantitation, the "effective" absorptivity (ϵ) of the dye was independently measured by constructing a calibration curve with varying amounts of dye in a buffer solution containing the same concentration of wild-type CPMV as used in the attachment reactions. The experimental error for the quantitation of dye attachment is $\pm 10\%$ of the value reported. The intact nature of the derived particles was substantiated by sucrose gradient ultracentrifugation and size-exclusion FPLC chromatography (SuperoseTM-6); nonspecific dye adsorption to the virus was ruled out by appropriate control experiments.

Nanogold labeling. Monomaleimido-Nanogold, (Nanoprobes, Inc., 6 nmol) was dissolved in isopropanol (10 μ L). The resulted dark brown solution was mixed with the cysteine mutant virus (60 μ g) in 0.1 M potassium phosphate buffer (pH 6.0, 90 μ L, containing 10 mM tris(2-carboxyethyl)phosphine). After 20 h at 4 °C, the reaction mixture was purified by passage through size-exclusion gel as described above. The product was collected as a colorless solution (200 μ L) with virus concentration = 0.45 mg/mL, which was used directly for the cryo-EM study.

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Table of Contents Entry This section has been revised.

Virus particles can be treated as very large molecules. Cowpea mosaic virus, an icosahedral nucleoprotein structure (see transmission electron microscopy image), is available in gram quantities and participates in selective organic reactions with thiol-specific organic and inorganic reagents. A mutant virus, engineered to display 60 thiol groups on the exterior surface of the particle, was found to be highly reactive. X-ray crystallography and cryo-electron microscopy were used to validate the site-specific chemical reactivity of the coat protein. These results set the stage for the use of icosahedral plant viruses in applications ranging from catalysis to materials science.



Keywords: viruses, dendrimers, electron microscopy, polyvalency

Viruses may help make microscopic robots
<http://www.upi.com/print.cfm?StoryID=29012-052646-7431r>

Scripps

UPI Science News
Published 1/29/2002 6:54 PM

LA JOLLA, Calif., Jan. 29 (UPI) -- Viruses with molecules of gold and antibodies studded on their surfaces may one day invade tumors in pinpoint cellular surgery and help assemble electronic wires thinner than visible light wavelengths for handheld supercomputers.

Researchers at Scripps Research Institute in La Jolla, Calif., have discovered a way to attach molecules to the surface of a virus -- tacking on anything from metal to vitamins.

"We can attach anything we want to the surface of the virus," said researcher Jack Johnson.

Scientists hope to use viruses as microscopic robots with programmable chemistry, genetically modifying the germs to accept different molecule types in patterns on their surfaces.

One particularly tantalizing possibility scientists are investigating on behalf of the U.S. Naval Research Laboratory in Washington is building circuits of electrically conducting molecules on viral surfaces to form molecular computers.

"You can, in principle, determine the type of assembly you get by programming the building blocks," said researcher M.G. Finn.

The scientists experimented with a plant germ known as cowpea mosaic virus, which resembles a spiky soccer ball in shape. The virus, which is completely harmless to humans, is only 30 nanometers in diameter -- more than 30,000 times thinner than a human hair. The viral shell is made of 60 identical proteins and is remarkably stable in terms of temperature and acidity.

"They're very beautiful structures to look at," Finn said. "What really takes my breath away is how much information and function is encoded into these structures. That kind of information is something we really don't know how to create ourselves yet."

The scientists are taking advantage of more than a billion years of nature's handiwork with their research. They genetically modified the virus, essentially making its interiors and exteriors chemically stickier. So far, the researchers have attached everything from fluorescent dyes and gold clusters to antibodies, sugars and vitamin B to the germ's surfaces.

Finn said up to 60 molecules can be attached to the virus outer surface. It is possible to genetically program the virus to make it differentially sticky -- for instance, the upper half can have metal particles tightly packed onto it while the bottom has antibodies widely spaced apart on it.

"It may be a lot more effective to pack 60 antibodies onto a surface and let them do their work," Finn said in an interview with United Press International.

The viral particles also show the as-yet unexplained ability to spontaneously self-assemble into lines that intersect at right angles on silicon surfaces -- a talent no doubt of interest to the computer industry. If loaded with metal, the viruses could robotically assemble molecular wires.

"The goal is to give each virus particle a function that would be useful in an electronic circuit," Finn said.

However, molecular electronics may be years away, Finn said. More immediate applications may be in using the viruses to surgically target tumors that leave surrounding cells untouched, with antibodies that latch onto cancer cells studding the outside of the virus and chemotherapy drugs lining the inside of the germ.

The scientists also look forward to using the virus to help examine biochemicals that are previously unviewable.

"I think the work elegantly demonstrates the ability to use viral protein cages as scaffolding for directed chemical reactions," said virologist Mark Young at Montana State University in Bozeman. "This work represents a milestone, since

it suggests that assembled virus particles can be treated as chemically reactive surfaces that are potentially available to a broad range of organic and inorganic applications."

The researchers reported their findings in the European scientific journal *Angewandte Chemie*.

(Reported by Charles Choi in New York.)

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